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Bacterial Etiology and Antimicrobial Resistance Profile of Urinary Tract Infections among Children in a Tertiary Care Health Setup

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Abstract: To study the bacterial etiology and antimicrobial resistance profile of urinary tract infections among children in a tertiary care health setup. A total of 300 children up to 14 years of age who presented to the pediatric outpatient department or were admitted to inpatient ward with a clinical diagnosis of urinary tract infections (UTI) were included in the study. Appropriate samples were collected and processed according to standard laboratory methods for isolation of bacterial pathogens and detecting their antimicrobial susceptibility pattern. Prevalence of UTI among pediatric population in our hospital was found to be 17%. Males were more affected than females. Majority (58.8%) of the culture positive isolates were Gram negative viz *E. coli* (52.9%) and *Klebsiella spp.* (5.2%). Among Gram positive isolates incidence of *Staphylococcus aureus* and *Enterococcus spp.* were found to be 11.7% and 29.4% respectively. *E. coli* (52.9%) was the most common isolate showing 88.8% sensitivity to imipenem, meropenem and 77.7% to nitrofurantoin and gentamicin. Pediatric uropathogens are showing high rate of drug resistance.
Keywords: Urinary tract infections, Children, *E. coli*, Pediatric age group

INTRODUCTION

Urinary tract infection (UTI) is one of the most commonly encountered clinical entities by the pediatrician both in the community and hospital settings. Around the globe approximately 8% of girls and 2% of boys have at least one episode of UTI by the time they turn seven and within a year 12-30% of them experience a recurrence [1].

In children poor toilet training, infrequent micturition, phimosis, incomplete bladder emptying, nocturnal enuresis put the child at risk of UTI [2]. The clinicians often miss the diagnosis as infant & children present with vague symptoms as nausea, vomiting, fever; urine cultures are not done thus, UTI is under reported. UTI if not treated can lead to pyelonephritis, renal damage and at times fatal bacteremia [3].

Prompt diagnosis and timely treatment may give relief from short-term symptoms and more importantly prevent potentially long-term complications. However, it is a very challenging job to establish a diagnosis in neonates, infants, toddlers and children; many are pre-verbal and collection of appropriate uncontaminated urine samples for culture is difficult [4].

The structurally and functionally normal urinary tract is usually considered as sterile [5]. Contamination by bowel flora may result in infection of the urinary tract if virulent organism is involved. In general, Gram negative bacteria belonging to the Enterobacteriaceae family account for 80-85% of etiology of UTI of which *Escherichia coli* (*E. coli*) is the leading causative organism followed by *Klebsiella spp*; other important Gram negative bacteria are *Citrobacter*, *Acinetobacter*, *Enterobacter*, *Pseudomonas* and *Proteus spp*. Among the Gram positive bacteria *Enterococcus spp.* and *Staphylococcus spp.* are the more common causative organisms of UTI [6-8].

Broad-spectrum antibiotics are often given in UTI empirically as the laboratory results of antimicrobial susceptibility testing take time, on an

average two to three days after sampling [9]. In recent times, antimicrobial resistance in members of *Enterobacteriaceae* has risen tremendously worldwide; nowadays global concern is the emergence of extended spectrum beta-lactamase (ESBL) producing organisms which was initially confined to the hospital settings, eventually these pathogens have emerged in community-onset UTIs also [10,11]. This trend amongst bacteria is of concern all around the globe and may undermine empiric therapy. It has been observed that there are many countries where prescription of antibiotics is unregulated and here in these places the antimicrobial resistance pattern of commonly isolated uropathogens is disturbing.

Various studies done in the past have shown that there are geographic variations in etiologic agents of UTIs and their resistance patterns to antibiotics [12, 13]. Therefore, knowledge about the local bacterial ethology and its antibiotic susceptibility pattern should be monitored for any change that might occur in time and to be updated the empirical antibiotic therapy of UTI. Thus the present study is planned to know the local antimicrobial resistance pattern among uropathogens and also for evidence based recommendations in empirical antibiotic treatment of UTI among children.

MATERIALS AND METHODS

Study design

The present study was a hospital based cross-sectional prospective observational study to determine the bacterial etiology and antimicrobial resistance profile of UTI. The study was done in Department of Microbiology of T.S. Mishra Medical College and Hospital, Lucknow during the period from July–August 2017.

Study Population

Children up to 14 years of age who presented to the paediatric outpatient department or were admitted to inpatient ward with a clinical diagnosis of UTI were included in the study. On the basis of current population of children less than 14 years of age 300 children were selected randomly.

Selection Criteria

The children were included in the study according to following criteria.

Inclusion criteria

- Children who presented with UTI symptoms and whose urine samples showed significant bacterial growth ($\geq 10^5$ CFU/ml)

Exclusion criteria

- Urethral catheterization
- Anatomical anomalies of urinary tract
- Immunocompromised states
- Oral antibiotic use in the past 3 days and intramuscular use in 28 days.
- Samples which grew more than one type of organism were considered as contaminated

Data Collection

Demographic (age, sex), clinical conditions data (history of antibiotic usage, duration of hospitalization, and history of fever) and other relevant information about the participants were recorded on a pretested preform.

Sample collection

Approval from the institutional ethical committee was obtained prior to commencement of the study. Informed consent was obtained from parents/guardians of all participants before specimen collection. Epidemiological information was obtained by interviewing the parents/guardian of the participants at the time of submission of sample. According to the age of the child, urine sample was collected by appropriate recommended method. In the age group of 2 months to 2 years: urine was collected by supra-pubic aspiration. In children of age 2 years to 5 years, attendants of the patients were counselled regarding the collection of clean catch mid-stream urine and a sample of 30-50 ml was requested. While in children who were of age 5 to14 years, urine was collected by clean catch mid-stream urine. For routine urine examination 10-15ml was taken and for urine culture 5ml was taken. Samples were collected in a sterile wide mouthed plastic container (capacity 100 ml) with a lid provided by the department.

Sample processing

The sample was processed as mentioned below:

Inoculation of specimens

The sample was processed immediately after collection. The urine sample was put in the refrigerator, if not processed within one hour of receipt. The culture was done under standard protocols using semi quantitative method. Each specimen was directly inoculated onto blood agar and MacConkey agar using a sterile standard calibrated wire loop (0.001ml), and streaked culture plates were incubated at 37^{o}C aerobically for 24hrs. Prolonged incubation was done for further 24 hours in case of no growth. Each pure colony was counted as colony forming units (CFU). Cultures with colony counts greater than 10^5CFU/ml, for single isolated bacteria were considered significant and positive for UTI. In cases of no growth after 48 hours of incubation the urine was interpreted as sterile whereas insignificant growth was reported in cases of colony count $<10^5$CFU/ml. Mixed growth of two or more organisms was considered as contamination. After culture was done approximately

5-10 ml of urine was centrifuged at 2000 rpm for 5 min. A wet preparation of the sediment was prepared and examined microscopically under high power objective for detection of pus cells, epithelial cells, crystals, fungal elements and RBCs. Presence of any bacteria per high power field (hpf) and pyuria (more than 5 white blood cells (WBCs) per hpf) was regarded as significant and suggestive of UTI.

Identification of bacterial isolates

Identification of bacterial isolates was done using colony characteristics on blood agar, MacConkey agar and Gram staining of bacteria and biochemical tests were performed accordingly like for Gram positives; catalase, coagulase, bacitracin, novobiocin and for Gram negative: motility, glucose and lactose fermentation, indole, Simmon's citrate, urease tests following standard microbiological methods [21]. The culture method, identification procedures and reporting system were similar throughout the periods when samples were processed

Antibiotic susceptibility testing

The antibiotic susceptibility testing was performed by using the Kirby-Bauer disc diffusion method on Muller Hinton agar using the criteria of standard zone sizes of inhibition to define sensitive, intermediate or resistance to different antimicrobials. The following antibiotic discs Ampicillin (25µg), Piperacillin-tazobactam (100/10µg), Chloramphenicol (30µg), Nitrofurantoin (300 µg), Gentamicin (10µg), Ciprofloxacin (5µg), Levofloxacin (5µg), Norfloxacin (5µg), Ceftazidime (30µg), Cephalothin (30µg), Ceftriaxone (30 µg), Doxycycline (30µg), Trimethoprim-Sulfamethoxazole (TMP-SMX) (25µg), and Tetracycline (30 µg) Imipenem (10 µg), Meropenem (10 µg) procured from Hi-media Labs, Mumbai, India were used. *E. coli* ATCC 25922 and *Staphylococcus aureus (S. aureus)* ATCC 25923 were used as reference strain for the standardization of antibiotic susceptibility testing [14].

Statistical Analysis

The data was collected and recorded in MS Office Excel Sheet. The chi-square test was applied to determine the association of isolation among different types of cases. P value <0.05 was considered as statistically significant.

Ethical consideration

Ethical clearance was obtained from the Institutional Ethical Committee for the study. The informed consent form was duly filled and signed by the guardian.

RESULTS

In the present study 300 children who attended the OPDs/admitted to the IPDs with a clinical diagnosis of UTI in our tertiary care hospital of Northern India were included in the study. Informed consent was obtained before enrolling the participant in the study. Out of the 100 participants who were enrolled, 108(36%) were females and 192(64%) were males. The age group distribution is shown in Table 1.

Among total clinical specimens, 51(17%) were found with growth of at least one significant pathogen confirming urinary tract infection (UTI). Male (64%) were most affected group of patients in both inpatient and outpatient department (p< 0.05). Maximum number of cases was found in the children of age group 10 to 14 years (Table 1).

Table-1: Distribution of confirmed cases of UTI as per sex, age and department (n = 300)

Socio-demographic Characteristics		Total	UTI
Sex	Male	192	34
	Female	108	17
Age group	<1 year	32	-
	1-4 years	49	6
	5-9 years	93	15
	10-14 years	126	30
Department	Outpatient	69	6
	Inpatient	231	45

Majority (58.8%) of the culture positive isolates were Gram negative viz *E. coli* (52.9%) and *Klebsiella spp.* (5.2%). Among Gram positive isolates incidence of *Staphylococcus aureus* and *Enterococcus spp.* were found to be 11.7% and 29.4% respectively. *E. coli* (n =27, 52.9%) was the most common organism isolated from urinary tract infections in pediatric group in this study.

Table-2: Various bacteria isolated from cases of UTI (n= 51)

Organism		Number
Gram negative bacteria	*Escherichia coli*	27
	Klebsiella spp.	3
Gram positive bacteria	*Enterococcus spp.*	15
	Staphylococcus aureus	6

E. coli showed 88.8% susceptibility to imipenem and meropenem, 77.7% to nitrofurantoin and gentamicin, 55.5% to cotrimoxazole, 44.4% to ciprofloxacin, levofloxacin, ceftazidime, ceftriaxone and piperacillin-tazobactam. The next common organism was *Enterococcus spp*. which showed a 100% susceptibility to imipenem, nitrofurantoin and vancomycin, 60% to ciprofloxacin and levofloxacin, 40% to doxycycline and chloramphenicol and 20% to ampicillin, tetracycline and trimethoprim-sulfamethoxazole (Table 3).

Table-3: Antimicrobial drug susceptibility pattern of uropathogens

	E. coli (n=27)	*Klebsiella spp.* (n=3)	*Staphylococcus aureus* (n=6)	*Enterococcus spp.* (n=15)
Ampicillin	22.2%	0%	50%	20%
Piperacillin-tazobactam	44.4%	100%	33.3%	0%
Nitrofurantoin	77.7%	66.6%	100%	100%
Gentamicin	77.7%	33.3%	50%	0%
Ciprofloxacin	44.4%	33.3%	33.3%	60%
Levofloxacin	44.4%	66.67%	50%	60%
Norfloxacin	22.2%	0%	0%	0%
Cephalothin	22.2%	0%	0%	40%
Ceftazidime	44.4%	33.3%	50%	0%
Ceftriaxone	44.4%	33.3%	50%	0%
Doxycycline	22.2%	100%	83.3%	40%
Tetracycline	33.3%	33.3%	66.7%	20%
Chloramphenicol	22.2%	33.3%	33.3%	40%
Cotrimoxazole	55.5%	0%	0%	20%
Imipenem	88.8%	100%	100%	100%
Meropenem	88.8%	100%	66.6%	0%
Vancomycin	--	--	100%	100%

DISCUSSION

In pediatric population, UTI is a probably one of the most common bacterial infection. The clinical features in most cases may be nonspecific or vague; not referable to the urinary tract making diagnosis difficult. In recent times, UTI has become more relevant in childhood morbidity as there may be acute and/or chronic consequences of this infection leading to renal damage. In this study UTI was diagnosed in 17 children giving a prevalence of 17%, which is quite comparable with other studies [15-17].

E. coli (52.9%) was the predominant pathogen isolated from urinary tract infections in pediatric group in this study. Similar rates have been reported in various studies conducted in nearby hospitals as well as from other countries [18, 19].

We found in this study that significantly more males (64.7%) with UTI than females which does not corroborate with other studies. This could be explained by the fact that this being a hospital- based study does not give a true picture of pediatric population in general; the exact prevalence cannot be extrapolated. Concurrently the maximum numbers of UTI cases were in children of age group 10-14 years and more inpatients were found with UTI ($p < 0.05$) as also reported in other similar studies.

Uropathogens are showing trend of increase in the antimicrobial resistance pattern throughout the world which can be explained by the fact that drugs are easily available over the counter in many countries, where prescription is not monitored and regulated leading to misuse of many antimicrobials. This problem is especially of concern in the developing countries as majority of patients often are unable to afford the consultation of a physician or have a laboratory analysis made

Increasingly recognized as a notorious uropathogen showing resistance to many of the available antibiotics, *E. coli* is of global concern worldwide. In a study done in Croatia, Ilić et al showed that the most frequent isolate was *E. coli* (67.7%) showing resistance to ampicillin (69.5%), amoxicillin/clavulanic acid (3.5%), cephalexin (6.6%), TMP-SMX (27.5%) and nitrofurantoin (0.4%) [20]. Resistance among *E. coli* was highest for TMP-SMX (24%) but lowest for nitrofurantoin (< 1%) was shown in a study conducted in United States of America including outpatients with UTI [21]. All isolates of *E. coli* were found to be resistant to ampicillin in a study from Iran but showed high sensitivity to nitrofurantoin, gentamicin, cefotaxime, and ciprofloxacin [22]. In our hospital setting *Enterococcus spp*. was found in higher number other than *E. coli* which showed a 100% susceptibility to imipenem, nitrofurantoin and

vancomycin, 60% to ciprofloxacin and levofloxacin, 40% to doxycycline and chloramphenicol and 20% to ampicillin, tetracycline and cotrimoxazole.

Based on the findings in the present study, for Gram negative bacilli the highest resistance to amoxicillin and highest sensitivity to imipenem, meropenem and gentamicin and in Gram positive cocci the highest resistance to ampicillin and TMP-SMX and highest sensitivity to linezolid and vancomycin were seen

There were some limitations of this study. As it was a project of short duration, results should be reevaluated in larger studies; generalization of the result to all of the patients with UTI cannot de done. Furthermore, patients were selected from a referral and tertiary care teaching hospital, possibility of potential bias cannot be ruled out.

CONCLUSION

Prevalence of UTI among pediatric population in our hospital was found to be 17%. Males were more affected than females. *E. coli* was the most common isolate showing sensitivity to imipenem, meropenem and nitrofurantoin.

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